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# A green method for the quantification of plastics-derived endocrine disruptors in beverages by chemometrics-assisted liquid chromatography with simultaneous diode array and fluorescent detection

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ABSTRACT

The aim of this study was to develop a novel analytical method for the determination of bisphenol A, nonylphenol, octylphenol, diethyl phthalate, dibutyl phthalate and diethylhexyl phthalate, compounds known for their endocrine-disruptor properties, based on liquid chromatography with simultaneous diode array and fluorescent detection. Following the principles of green analytical chemistry, solvent consumption and chromatographic run time were minimized. To deal with the resulting incomplete resolution in the chromatograms, a second-order calibration was proposed. Second-order data (elution time-absorbance wavelength and elution time-fluorescence emission wavelength matrices) were obtained and processed by multivariate curve resolution-alternating least-squares (MCR-ALS). Applying MCR-ALS allowed quantification of the analytes even in the presence of partially overlapped chromatographic and spectral bands among these compounds and the potential interferents. The obtained results from the analysis of beer, wine, soda, juice, water and distilled beverage samples were compared with gas chromatography-mass spectrometry (GC-MS). Limits of detection (LODs) in the range 0.04–0.38 ng $mL^{-1}$ were estimated in real samples after a very simple solid-phase extraction. All the samples were found to contain at least three EDs, in concentrations as high as 334 ng $mL^{-1}$.



## 1. Introduction

According to the International Program on Chemical Safety (IPCS), an endocrine disruptor (ED) is “an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations” [1]. More than 87,000 compounds of diverse chemical nature and origin are listed as EDs by the U.S. EPA Endocrine Disruptor Screening Program (EDSP), and are classified into several categories: hormones, pharmaceuticals, personal care products, industrial chemicals, pesticides, and combustion byproducts [2]. Phthalate esters (PAEs), alkylphenols (APs) and bisphenol A (BPA) are EDs of particular interest because of their extensive use, mainly in the production of food contact materials, but also in cosmetics, personal care products, medical devices and building materials [3]. In this work some of the most widely spread EDs belonging to these categories are studied, namely, BPA, 4-octylphenol (OP), 4-nonylphenol (NP), diethyl phthalate (DEP), dibutyl phthalate (DBP) and diethylhexyl phthalate (DEHP) (Fig. 1).

PAEs are used as plasticizers in the production of polyethylene, polyvinyl chloride, and other synthetic materials [4]. Since PAEs are not covalently bound to plastics, they can be transferred to the environment, or leak from packing material into food and beverages [5]. On the other hand, APs are derived from the

*Abbreviations:* AJ, apple juice; APs, alkylphenols; BPA, bisphenol A; BSTFA, *N,O*-bis (trimethylsilyl)trifluoroacetamide; Cac, cachaça; ACN, acetonitrile; DAD, diode array detector; DBP, dibutyl phthalate; DEHP, diethylhexyl phthalate; DEP, diethyl phthalate; EDs, endocrine disruptors; EJCR, elliptical joint confidence region test; FLD, fluorescence detector; GC-MS, gas chromatography-mass spectrometry; IUPAC, International Union of Pure and Applied Chemistry; LB, lager beer; LC, liquid chromatography; LC-DAD, elution time-absorbance wavelength matrices; LC-FLD, elution time-fluorescent emission wavelength matrices; LOD, limit of detection; LOQ, limit of quantification; LS, lime soda; MCR-ALS, multivariate curve resolution-alternating least-squares; MeOH, methanol; MP, mobile phase; MW, mineral water; nd, not detected; NOAEL, No Observed Adverse Effect Level; NP, nonylphenol; OP, octylphenol; PAEs, phthalate esters; RMSEP, root-mean-square error of prediction; RW, red wine; SB, stout beer; Sch, schnapps; SML, specific migration limit; SPE, solid-phase extraction; TDI, tolerable daily intake; TMCS, trimethylchlorosilane; TW, tonic water; WW, white wine

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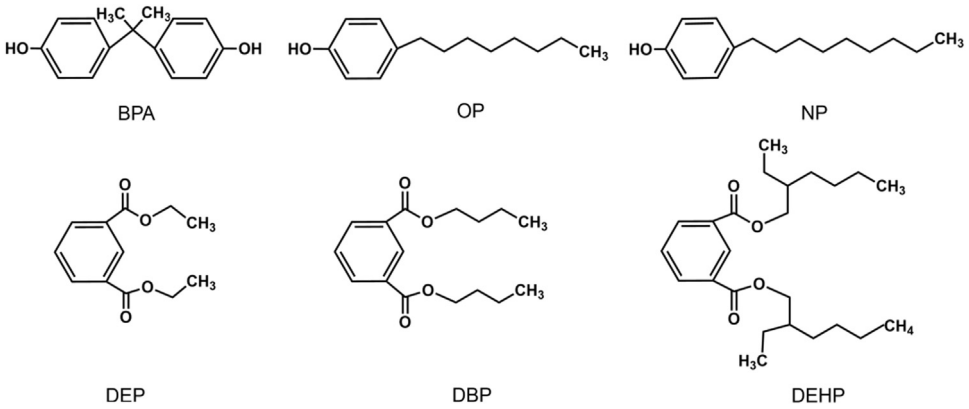


**Fig. 1.** Structures of the studied EDs.

degradation of alkylphenol ethoxylates, which are surfactants commonly employed in the manufacture of detergents, soaps, paints and other domestic and industrial products [6]. NP is also used as an additive to improve plastic properties [7]. BPA is utilized in the production of epoxy resins, applied as internal coatings of food and beverage cans, and as a monomer in the synthesis of polycarbonate based food contact materials [8].

Several biomonitoring studies have reported a widespread human exposure to PAEs, APs and BPA [9,10]. Even though routes of exposure may vary, diet is always considered the major source of intake [11]. Moreover, the presence of the studied analytes in food and beverages not only represents a health hazard, but also damages product quality, since EDs are a source of carbon for microorganisms that may negatively impact product taste and odour [12]. Therefore, it is essential to develop analytical methods for detecting and quantifying these compounds in a wide variety of food and beverage samples. According to the literature, the most commonly used methods involve liquid chromatography (LC), either equipped with a diode array detector (DAD) or a mass spectrometer [13,14] and gas chromatography-mass spectrometry (GC-MS) [15,16]. Since EDs can be found in beverages at concentrations as low as parts per trillion [17,18] reported methodologies often include a clean-up/preconcentration step prior to instrumental analysis, such as liquid-liquid extraction [19], solid-phase extraction (SPE) [20], and different variants of liquid-liquid micro-extractions [21].

With the purpose of developing a green analytical methodology, i.e. not requiring intense sample pretreatment and minimizing the use of organic solvents [22], a fast and simple method involving a second-order calibration for the quantification of EDs in beverages is here proposed. Analysis was performed through LC with simultaneous measurement of elution time-absorbance wavelength (LC-DAD) and elution time-fluorescent emission wavelength (LC-FLD) second-order data. In this case, instead of pursuing baseline resolution of the analytes, chromatographic conditions were set in order to minimize both solvent usage and experimental time. While dual detection permits the selection of the most appropriate signal for each analyte, second-order calibration enables resolution of overlapping bands and analyte quantification in the presence of interferents. Second-order data was processed by multivariate curve resolution-alternating least-squares (MCR-ALS) [23].

Due to the different chromatographic retention properties of the studied EDs, a simple elution gradient (see below) was applied to shorten the run time. After an easy SPE with C18 membranes, the method was successfully applied to the quantification of the studied plastics-derived EDs in twelve drinks, including beer, wine, soda, juice, water and distilled beverages, and a comparison with a GC-MS method was carried out.

## 2. Experimental

### 2.1. Apparatus

Chromatographic analysis was carried out on an Agilent 1200 liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a quaternary pump operating at 1.5 mL $min^{-1}$, a fluorescence detector (FLD) irradiating at 226 nm and collecting emission spectra from 295 to 350 nm and a DAD set at a wavelength range from 200 to 300 nm. Both detectors were connected in series, recording absorbance and fluorescence data simultaneously. A Rheodyne injector with a 20.0 μL loop was employed to inject the sample onto a Poroshell 120 EC C18 column (4.6 mm × 50 mm, 2.7 μm particle size). The data were collected using the software HP ChemStation for LC Rev. HP 1990–1997.

GC-MS was performed using a Shimadzu GC MS-QP2010 Plus gas chromatograph (Kyoto, Japan), equipped with an automatic injector and a Supelco SPB-1 capillary column (30 m × 0.25 mm, $d_f$ 0.25 μm). For quantitative determinations, the detector was operated in selected ion-monitoring (SIM) mode. Data acquisition and integration were carried out with the LabSolutions chromatography software.

### 2.2. Reagents and solutions

All reagents were of high-purity grade and used as received. BPA, OP, DEP, DBP and DEHP were purchased from Sigma-Aldrich (St. Louis, MO, USA). NP was provided by Fluka (Buchs, Switzerland). Methanol (MeOH) and acetonitrile (ACN) were obtained from Merck (Darmstadt, Germany) and ethyl acetate by Carlo Erba (Milan, Italy). Ultrapure water was obtained by a Milli Q apparatus (Millipore, Molsheim, France).

MeOH stock solutions of BPA, OP, NP, DEP, DBP and DEHP of about 1000 mg $L^{-1}$ were prepared and stored in dark flasks at 4 °C. From these solutions, more diluted MeOH solutions (2.00–10.0 mg $L^{-1}$) were obtained.

Empore C18 SPE disks, *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) were supplied by Supelco (Bellefonte, PA, USA).

PAEs are ubiquitous laboratory contaminants. Therefore, sample contact with plastic materials during storage, transfer and measurements was avoided. All glassware used for the analysis were soaked with a mixture of potassium dichromate, sulfuric acid and water for 24 h, carefully rinsed with tap and Milli-Q water, and finally with methanol. Because of the toxicity of chromium solutions, minimal amounts of the potassium dichromate/sulfuric acid mixture were used. In addition, care was taken in handling the latter mixture, and all rinses were collected for proper disposal.

With the exception of calibrated flasks, the material was dried at 100 °C overnight before use. The good recoveries obtained in validation samples (see below) suggest that these precautions were effective.

### 2.3. Calibration and validation procedures

A calibration set was constructed by preparing 12 samples, following a randomized design, with concentrations in the ranges 0–50 ng $mL^{-1}$ for BPA, NP and OP, and 0–200 ng $mL^{-1}$ for DEP, DBP and DEHP. A validation set of 10 randomized samples was also prepared in the corresponding calibration ranges. Solutions of both sets were prepared as follows: aliquots of MeOH standard solutions of the analytes were placed in 5.00 mL volumetric flasks and the solvent was dried under nitrogen. The flasks were then filled to the mark with a mixture of ACN: water (70:30 v/v). Finally, samples were filtered through a 0.22 μm nylon membrane and analyzed by LC.

The chromatographic analysis was performed using a mixture of water (solvent A) and ACN (solvent B) as mobile phase (MP). Prior to LC analysis, both solvents were filtered by vacuum through a 0.22 μm nylon filter. An elution gradient program was employed: 0–4.5 min, isocratic elution with 30% A:70% B, 4.5–6 min, linear gradient from 30% A:70% B to 7% A:93% B; 6–9 min, isocratic elution with 7% A:93% B. Finally, the MP composition was brought back to the initial conditions, and after a reconditioning period of 5 min, the next sample was injected, giving a total run time of 14 min per sample.

The detectors were set in the conditions previously described, and two sets of matrices were simultaneously collected: one for DAD (every 0.85 s, in the range 200–300 nm, each 0.5 nm), and one for FLD (every 0.85 s, in the range 295–350 nm, in steps of 1 nm). All matrices were saved in ASCII format, and transferred to a PC Sempron AMD microcomputer for subsequent computational treatment.

### 2.4. Beverage samples

Twelve real samples, including mineral water, juice, soft drinks, wine, beer and distilled beverages were purchased from local markets and stored at 4 °C before sample preparation. Carbonated drinks and beers were degassed in an ultrasonic bath for 5.0 min, and in the case of alcoholic beverages, ethanol was removed by means of a rotatory evaporator, to ensure a maximum recovery in the subsequent sample treatment. All samples were then filtered with 0.45 μm nylon membranes and preconcentrated through SPE, using C18 SPE disks. The C18 membranes were conditioned with 0.75 mL of MeOH and the extraction of up to 40 mL of sample was carried out, maintaining a flow rate in the optimum range for maximum breakthrough volume (10–30 mL $min^{-1}$) [24]. The retained compounds were then eluted with approximately 0.75 mL of MeOH into a 1.00 mL volumetric flask, and completed to the mark with MeOH. For LC analysis, 25–250 μL of the eluate were dried under nitrogen, reconstituted with 250 μL of a mixture of ACN: water (70:30 v/v) and subjected to the same chromatographic analysis as the test samples. In this way, the maximum preconcentration factor achieved was 40.

### 2.5. GC-MS

The obtained results for the real samples were compared with GC-MS, following a modified version of the procedure suggested by Ballesteros et al. [15]. Real samples were treated in a similar way to the above description, but in this case the preconcentration factors ranged from 40 to 400. Since BPA, OP and NP are not volatile enough to be analyzed by this technique, samples were derivatized prior to injection. The derivatization process was carried out as follows: the samples were dried under nitrogen and 30 μL of a 25:5 mixture of ethyl acetate: BSTFA/TMCS 1% were added. The vials were then sealed, homogenized by means of a vortex, and heated at 80 °C for 30 min. Finally, 3 μL of the derivatized samples were injected into the gas chromatograph.

Helium was employed as carrier at a flow of 1 mL $min^{-1}$. The injection port temperature was set at 250 °C. The ionization energy applied was 70 eV. An oven temperature gradient was employed to achieve resolution of the analytes. An initial oven temperature of 120 °C was held for one min, then a linear gradient from 120 °C to 230 °C was applied for 9 min, and finally, the oven temperature was kept at 230 °C for another 9 min. Scan mode was employed to identify the analytes, while selected ion mass monitoring mode was used for quantification (*m/z* 149 for DEP, DBP and DEHP, *m/z* 179 for NP, *m/z* 278 for OP and *m/z* 357 for BPA).

### 2.6. Chemometric algorithm and software

For a brief theoretical description of the applied algorithm (MCR-ALS), see Supplementary material. The routines employed are written in MATLAB 7.0. The algorithm was implemented using a new version of the graphical interface of the MVC2 toolbox [25] freely available on the Internet [26].

## 3. Results and discussion

### 3.1. General considerations

When developing a new analytical method under the green analytical chemistry principles, minimizing both solvent and energy consumption is extremely important [27]. To this end, fast and energy-efficient multi-analyte analysis, with minimal sample treatment and no derivatization are always preferred. Following these guidelines, chromatographic conditions were optimized to shorten the elution time as much as possible, instead of achieving baseline resolution of sample components. As a consequence of this approach, resolution between some analytes was lost, and a multi-way calibration was necessary to overcome the temporal overlapping present in the chromatograms. Considering the constitution of the analyzed samples and the possible presence of interferents, a second-order calibration was attempted with the purpose of attaining the second-order advantage, a property of second-order data that allows analyte quantification in the presence of foreign components not present in the calibration samples [28].

Since fluorescent detection generally shows higher sensitivity and selectivity, analytes with fluorescent properties, i.e. BPA, OP and NP, were quantified by FLD, while concentrations of DEP, DBP and DEHP, which show no significant luminescent properties, were detected by DAD. Calibration and validation concentration ranges for both sets of analytes were selected taking into account the importance of determining low levels of EDs in beverages. Therefore, no efforts were made to establish the upper concentrations of the corresponding linear ranges.

Different chromatographic conditions were tested, i.e. type and column length, MP constitution and flow rate. In order to achieve a high flow rate and decrease the run time, a C18 column with a length of 50 mm was chosen. Regarding MP, isocratic conditions are generally preferred, because the analysis becomes simpler, and there is no need of reconditioning periods. Thus, in preliminary experiments, isocratic MP compositions based on the experimental conditions described by Li et al. [29] and Ranjbari et al. [30] using ACN: water and MeOH: water systems were assayed. The high back pressure generated by MeOH: water mixtures due to

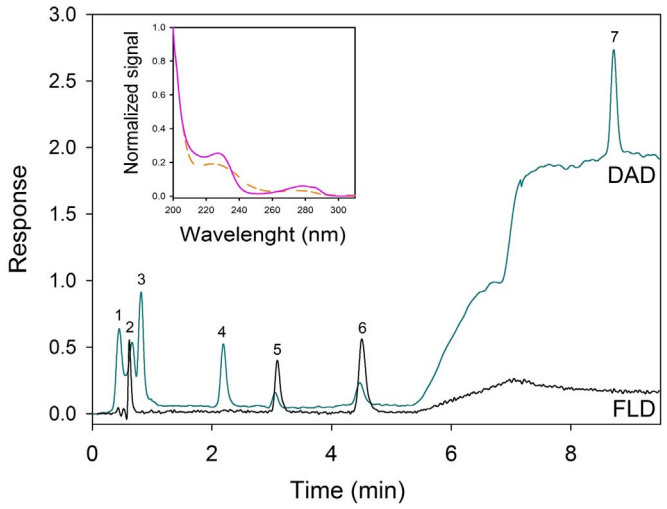


**Fig. 2.** DAD (black) and FLD (dark cyan) chromatograms of a synthetic sample. Peak numbers refer to (1) injection signal, (2) BPA, (3) DEP, (4) DBP, (5) OP, (6) NP, and (7) DEHP. Inset Normalized absorption spectra in acetonitrile-water (70:30, v/v) for BPA (pink solid line) and DEP (orange dashed line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

their high viscosity [31] precluded the use of fast flow rates and thus ACN: water mixtures were tested. However, owing to the varying nature and polarity of the analytes, suitable conditions for the separation of DEP, DBP and BPA caused large retention times for DEHP, leading to run times of up to 40 min. For this reason, an elution gradient was applied which significantly reduced the analysis time. The initial composition of the MP (ACN: water 70:30% v/v) was selected in order to achieve maximum resolution of the less polar analytes in a short time, i.e. 4–5 min. A linear gradient was then applied for 1 min, reaching an MP composition of high organic content. These conditions were maintained for 3 min, leading to a total run time of 8.5 min

Fig. 2 depicts both DAD and FLD chromatograms of a synthetic sample containing the studied analytes. As displayed in the DAD chromatogram, baseline resolution of the injection signal, BPA and DEP was not achieved under the applied experimental conditions. However, as was previously stated, this fact does not represent a problem when working with second-order calibration.

### 3.2. Calibration and validation samples

In order to validate the method, recoveries and figures of merit were calculated using spiked samples (validation samples) at several concentration levels.

Both LC-DAD and LC-FLD matrices for the calibration and validation samples were measured and subjected to a baseline correction algorithm, based on an asymmetric least-squares method [32]. DAD and FLD data were processed separately, owing to the delay time between the two modes of detection, which affects the temporal profiles of the analytes.

Different algorithms are able to deal with second-order data. However, matrices of chromatographic origin constitute a special case, since temporal profiles are usually not constant from run to run, i.e. the obtained three-way data arrangements are not trilinear [33]. In such cases, trilinear methods require to previously align the chromatograms by means of specialized software [34]. Still, such procedures are tedious and their efficiency cannot be guaranteed in the presence of interferents. A more convenient solution is the application of the MCR-ALS algorithm, which unfolds the three-way data into an augmented matrix to preserve the bilinearity property [33]. Therefore, in the present system, MCR-ALS was selected to process the data, and augmentation was performed in the mode in which trilinearity is lost, i.e. the temporal direction (column-wise). Augmented data matrices were built with each validation sample and all the calibration samples.

**Table 1**
Selected chromatographic/spectral ranges used for MCR-ALS data processing.

| | Time (min) | Wavelength (nm) |
|---|---|---|
| Fluorescence detector (FLD) | | |
| BPA | 0.50–0.85 | 290–350 |
| OP | 2.80–3.50 | 290–350 |
| NP | 4.15–5.00 | 290–350 |
| Diode array detector (DAD) | | |
| DEP | 0.50–1.30 | 200–300 |
| DBP | 1.85–2.55 | 200–300 |
| DEHP | 8.25–9.10 | 200–300 |

PAEs have very similar absorbance spectra, while BPA, OP and NP have almost the same fluorescence emission profile, meaning that working with full chromatograms would lead to nearly zero spectral selectivity. Therefore, it was necessary to apply the algorithm in selected time ranges, in such a way that each region only included analytes with different spectral profiles (Table 1).

The number of components in each temporal region was determined by principal component analysis [23], and the results obtained were in agreement with the number of components theoretically expected. The initial profiles employed to start the MCR-ALS fitting were obtained by estimating the so-called purest variables in the spectral domain. In order to drive the iterative procedure to chemically interpretable solutions, the following constraints were applied: (1) non-negativity in both modes, (2) unimodality in each sub-profile of the temporal mode and (3) correspondence between components and samples [23]. After convergence of the ALS optimization, analytes were identified by their spectral profiles and their quantification was performed through the corresponding pseudo-univariate calibration curves.

Predicted vs. nominal concentration plots for validation samples were constructed (Fig. 3A and B), and a good correlation was observed for all analytes.

The elliptical joint confidence region (EJCR) statistical test was performed to check the accuracy of the predictions. This test consists in verifying if the ideal point (slope = 1, intercept = 0) is included in the elliptical region of mutual confidence of the slope and intercept in the predicted vs. nominal concentration plot [35]. Fig. 3C shows that the theoretically expected point lies inside the elliptical regions for all the analytes, indicating the accuracy of the proposed methodology. In the case of BPA and DEP, the developed second-order calibration allowed their quantification with adequate accuracy and precision, even though their temporal profiles were highly overlapped.

Table 2 summarizes the figures of merit for the validation samples processed by MCR-ALS, calculated following a rigorous approach recommended by the International Union of Pure and Applied Chemistry (IUPAC) [36]. It should be noted that limits of detection (LODs), limits of quantification (LOQs), and root-mean-square error of predictions (RMSEPs) are lower in the case of fluorescence detection, as expected. Nevertheless, LODs obtained without preconcentration for DEP, DBP and DEHP, in the range 10–18 ng $mL^{-1}$, are more than acceptable.

### 3.3. Real samples

The proposed method was employed in the determination of the studied EDs in beverages bottled in plastic containers and plastic lined cans (e.g. juice, mineral water, soda, schnapps and cachaça), and beverages contained in glass bottles (e.g. wine and

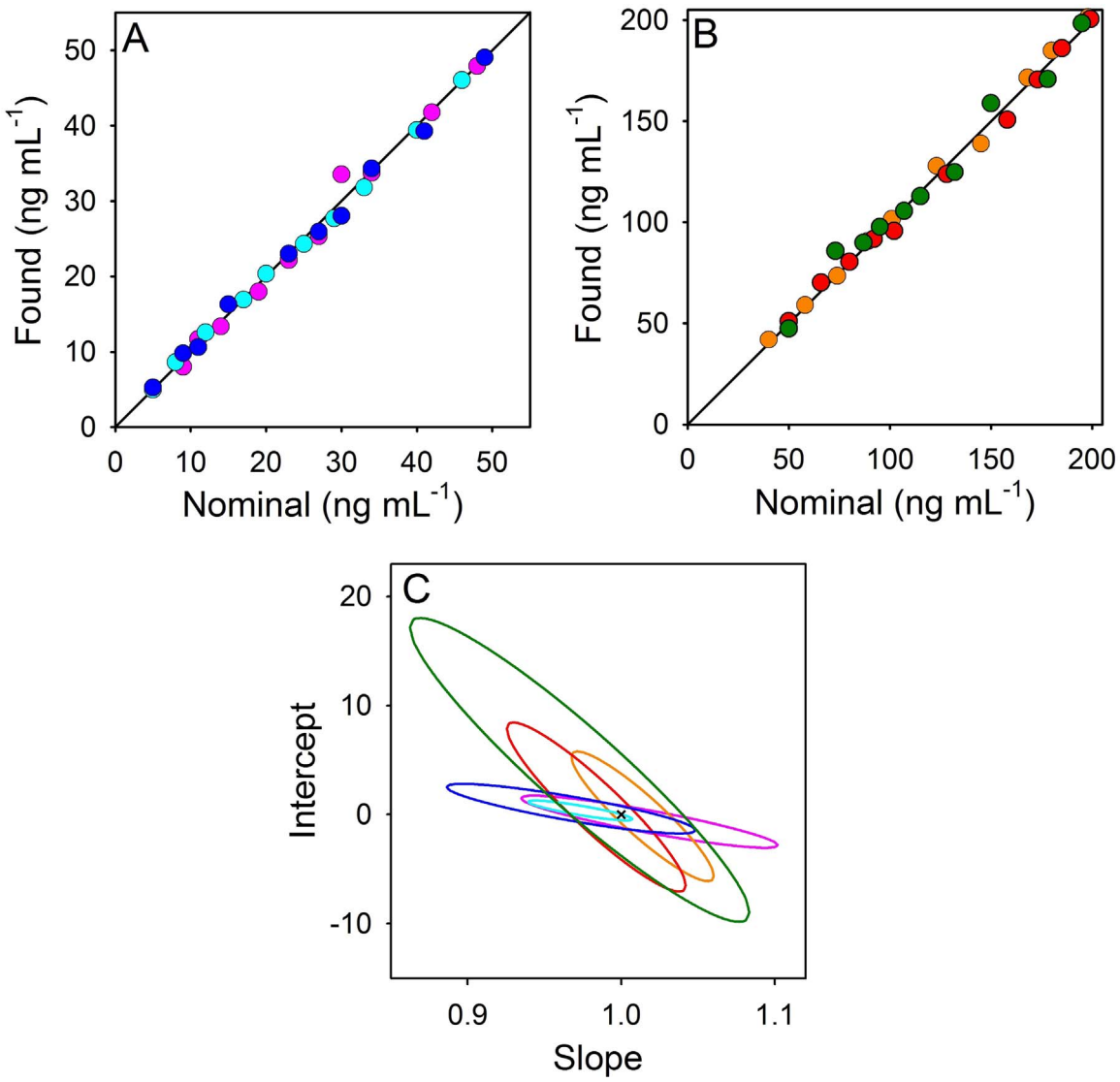


**Fig. 3.** (A) Plots of BPA (pink), OP (cyan) and NP (blue) predicted concentrations as a function of the nominal values in test samples (B), DEP (orange), DBP (red) and DEHP (green) predicted concentrations as a function of the nominal values in test samples and (C) elliptical joint regions (at 95% confidence level) for the slopes and intercepts of the regressions for the corresponding predictions. The black cross in the elliptical plots marks the theoretical (intercept=0, slope=1) point. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Table 2**
Figures of merit for the studied EDs measured with DAD or FLD in validation and real samples[a].

| | Fluorescence detector (FLD) | | | Diode array detector (DAD) | | |
|---|---|---|---|---|---|---|
| | BPA | OP | NP | DEP | DBP | DEHP |
| Validation samples | | | | | | |
| Calibration range (ng $mL^{-1}$) | 0–50 | 0–50 | 0–50 | 0–200 | 0–200 | 0–200 |
| $\gamma$ (mL $ng^{-1}$) | 3.1 | 5.4 | 7 | 2.7 | 2.7 | 4.1 |
| LOD (ng $mL^{-1}$) | 2.7 | 1.0 | 1.3 | 9.9 | 12.0 | 18.0 |
| LOQ (ng $mL^{-1}$) | 8.2 | 3.0 | 3.9 | 30.0 | 36.0 | 55.0 |
| RMSEP (ng $mL^{-1}$) | 1.4 | 0.7 | 1.0 | 3.4 | 3.8 | 6.2 |
| Real samples[b] | | | | | | |
| $\gamma$ (mL $ng^{-1}$) | 1.4 | 2.8 | 3.4 | 1.3 | 0.9 | 1.1 |
| LOD (ng $mL^{-1}$) | 0.11 | 0.04 | 0.06 | 0.25 | 0.38 | 0.30 |
| LOQ (ng $mL^{-1}$) | 0.35 | 0.12 | 0.18 | 0.75 | 1.10 | 0.89 |
| RMSEP (ng $mL^{-1}$) | 1.5 | 0.3 | 0.4 | 0.7 | 3.8 | 1.0 |

[a] $\gamma$, analytical sensitivity, LOD, limit of detection, LOQ, limit of quantification, and RMSEP, root-mean-square error of prediction.
[b] Preconcentration factor=40 (see text).

beer) but with a potential contact with plastics-derived EDs during production stages and transport.

In food samples, the EU Commission Regulation No 10/2011 establishes a Specific Migration Limit (SML) of 600, 10, 300 and 1500 ng $mL^{-1}$ for BPA, DEP, DBP and DEHP, respectively [37]. Although no SML values have been reported for OP and NP, a No Observed Adverse Effect Level (NOAEL) of 10 mg/kg/day [38] and a Tolerable Daily Intake (TDI) value of 5 μg/kg body-weight [39] have been suggested for OP and NP, respectively.

The EDs concentration values found in beverages are diverse and depend on the type of analyzed sample. Levels of ng $L^{-1}$ have been found in bottled water [18], while concentrations up to 30 ng $mL^{-1}$ have been encountered in soft drinks [17,18]. BPA concentrations up to 2 ng $mL^{-1}$ have been reported in wines [40] and high levels of PAEs (e.g. 1500 ng $mL^{-1}$) have been found in alcoholic drinks [41,42], which can be explained considering the extractive power of alcoholic solutions over the lipophilic PAEs [41]. Regarding OP and NP, to the best of our knowledge, there are no thorough surveys of their occurrence and concentrations in beverages.

In view of the reported concentrations of the studied analytes and the quantification limits here determined, it is apparent that a

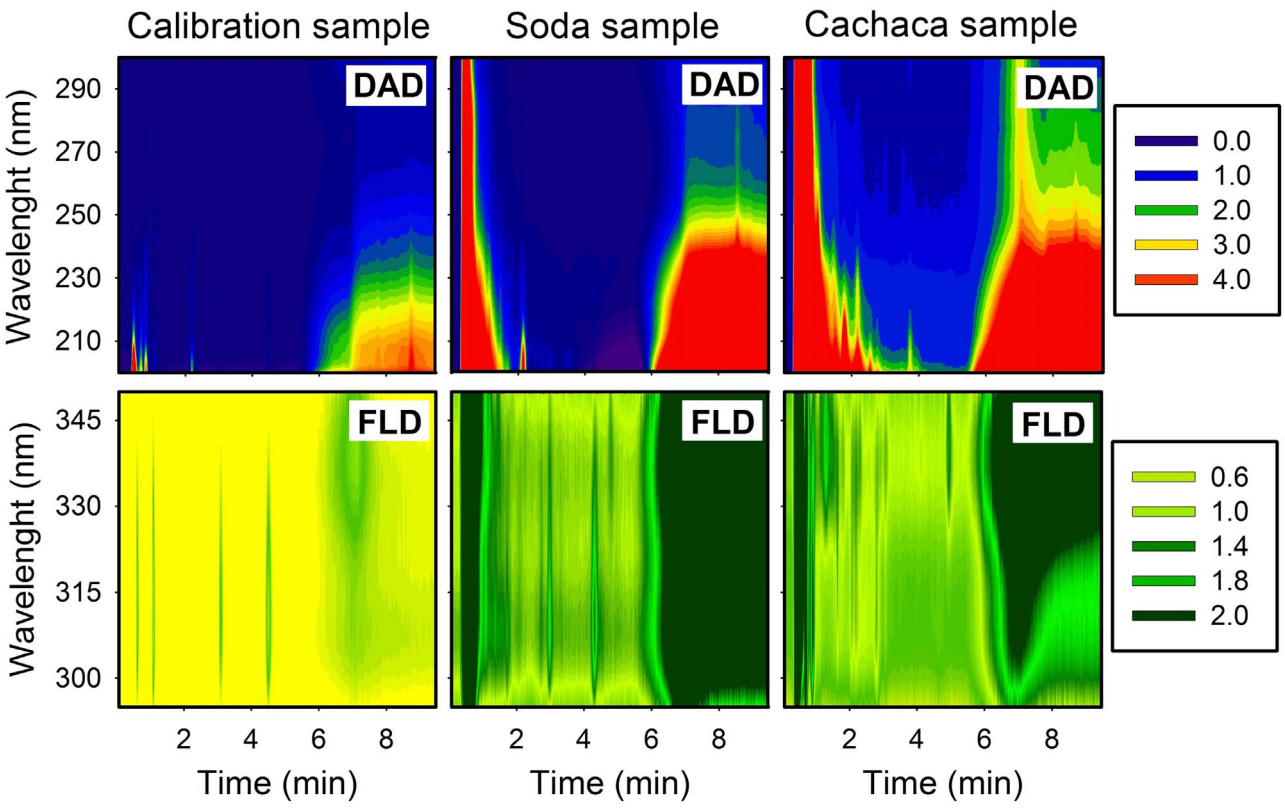


**Fig. 4.** Contour plots of LC-DAD and LC-FLD matrices for a calibration sample, and two beverage samples (from left to right).

preconcentration step is needed for their determinations in some of the investigated samples. SPE in C18 membranes was chosen because of its simplicity and low solvent consumption. Preconcentration factors ranged from 10 to 40, depending on the nature of the sample, and were achieved by changing the volume of beverage processed. It is important to point out that whereas large volumes (up to 100 mL) of toxic solvents (e.g. heptane and dichloromethane) are usually employed in the pretreatment of samples for ED determination [20,43,44], the method herein described only required the consumption of 2 mL of MeOH per sample.

In order to evaluate the efficiency of the extraction process, the recovery of analytes through the membrane was tested by the

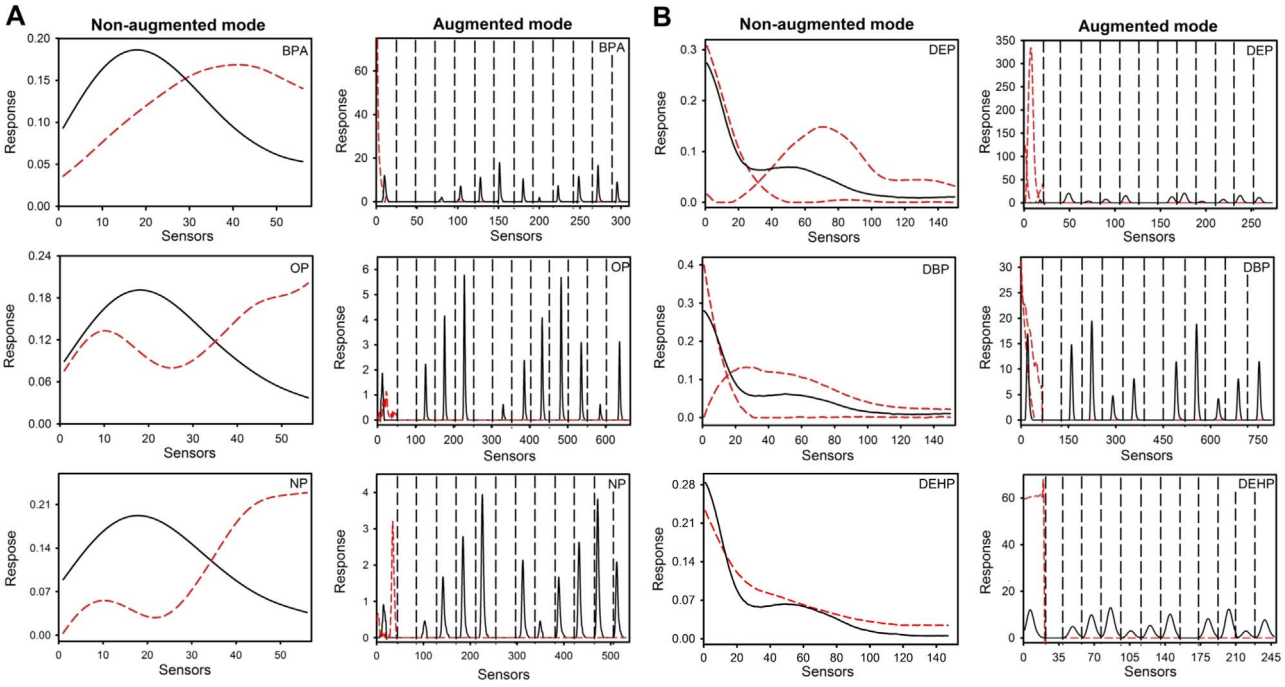


**Fig. 5.** For (A) BPA, OP and NP, and (B) DEP, DBP and DEHP: spectral profiles retrieved by MCR-ALS when processing a soda sample and the corresponding time profiles (the dotted vertical lines separate, from left to right, the studied sample and the successive calibration samples). In all plots, the solid black line indicates ED, and dashed red lines indicate background and interferents. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Table 3**
Determination of the studied EDs concentrations (ng $mL^{-1}$) in beverage samples using MCR-ALS and GC-MS[a].

| | BPA | | OP | | NP | | DEP | | DBP | | DEHP | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MCR-ALS | GC-MS | MCR-ALS | GC-MS | MCR-ALS | GC-MS | MCR-ALS | GC-MS | MCR-ALS | GC-MS | MCR-ALS | GC-MS |
| MW | 0.47 (3) | 0.46 | 0.51 (1) | 0.44 | 0.14 (1) | 0.14 | nd | nd | 27.3 (5) | 27.8 | 8.5 (8) | 8.7 |
| MW | 1.04 (2) | 1.18 | 0.44 (1) | 0.42 | 0.16 (2) | 0.13 | 4.9 (2) | 4.90 | 29.5 (8) | 30.8 | 12.1 (3) | 12.5 |
| LS | 1.48 (8) | 1.63 | 0.74 (2) | 0.86 | 0.18 (1) | 0.19 | 1.16 (8) | 1.20 | 13.9 (8) | 14.6 | 7.23 (5) | 6.80 |
| TW | 2.52 (2) | 2.72 | 0.96 (1) | 0.87 | 0.24 (1) | 0.26 | 39.2 (1) | 40.2 | 22.4 (1) | 21.2 | 14.2 (5) | 14.2 |
| AJ | 0.63 (2) | 0.68 | 4.4 (1) | 3.70 | 0.92 (4) | 1.01 | 2.8 (2) | 2.60 | 31.3 (2) | 31.7 | 21.6 (1) | 21.3 |
| LB | 0.98 (2) | 0.93 | nd | nd | 0.49 (4) | 0.45 | 4.7 (3) | 5.20 | 1.1 (1) | 1.3 | 18.2 (2) | 18.1 |
| SB | 55.6 (3) | 58.7 | 0.89 (4) | 0.93 | 0.77 (4) | 0.68 | nd | nd | 74.7 (9) | 73.1 | 16.6 (5) | 16.3 |
| RW | nd | nd | 0.77 (1) | 0.75 | 1.02 (3) | 1.03 | 56.0 (3) | 57.6 | 334 (1) | 343 | 80.3 (9) | 81.0 |
| RW | nd | nd | nd | nd | 0.58 (6) | 0.58 | 23.6 (4) | 23.2 | 39.6 (8) | 39.1 | 26.8 (1) | 26.5 |
| WW | nd | nd | nd | nd | 4.3 (2) | 4.3 | nd | nd | 32.4 (2) | 29.4 | 18.2 (5) | 18.7 |
| Sch | 11.8 (3) | 11.4 | nd | nd | 1.5 (2) | 1.6 | 4.7 (4) | 5.40 | 76.6 (6) | 74.9 | 28.0 (4) | 26.8 |
| Cac | 110 (1) | 107 | 6.78 (5) | 7.78 | 14.2 (2) | 13.2 | 25.8 (8) | 26.0 | 40.5 (1) | 42.8 | 140 (1) | 137 |
| $t_{ex}$[b] | 0.02 | | 0.20 | | 0.92 | | 1.81 | | 1.44 | | 1.13 | |
| $t_{crit}$[b] | 2.31 | | 2.37 | | 2.20 | | 2.31 | | 2.20 | | 2.20 | |

Abbreviations: AJ, apple juice; Cac, cachaça; LB, lager beer; LS, lime soda; MW, mineral water; nd, not detected; RW, red wine; SB, stout beer; Sch, schnapps; TW, tonic water; WW, white wine.

[a] Experimental standard deviations of duplicates are given between parentheses and correspond to the last significant figure.

[b] Calculated ($t_{ex}$) and tabulated ($t_{crit}$) values when a paired Student's $t$-test is applied at 95% confidence level and $n-1$ degree of freedom.

analysis of spiked samples at three different concentration levels. As indicated in the experimental section, in the case of alcoholic beverages, ethanol was removed prior to preconcentration. Recoveries in the range 90–100% were obtained for the investigated analytes in all samples.

After preconcentration, twelve real samples were investigated using the proposed second-order method. The effect of the presence of interferents in the recorded signal for real samples with respect to a typical calibration sample (without interferences) can be visualized in Fig. 4. The latter shows the corresponding contour plots of both LC-DAD and LC-FLD matrices for a calibration sample and two selected beverages. In fact, all analyzed samples contained interferents coeluting with at least one of the analytes. Such conditions precluded the quantification of the analytes by means of zero-order calibrations, and the second-order advantage became essential to separate the EDs signal from those of the interferents.

Real samples were processed with MCR-ALS, employing the same initialization and restrictions as the validation samples. Additionally, a correspondence constraint was applied concerning the potential interferences, i.e., their profiles in the augmented mode were forced to be zero in the calibration samples. In this way, the MCR-ALS algorithm was able to retrieve satisfactory spectral profiles, as can be observed in Fig. 5.

Table 3 summarizes the concentrations found for the studied EDs following the proposed methodology and those obtained with GC-MS. The two methods were compared through a paired Student's $t$-test. The $t$ values obtained for $n-1$ degrees of freedom (where $n$ is the number of evaluated levels) at a 95% of significance are smaller than the corresponding tabulated values, suggesting that there are no significant differences in the concentrations determined by both methods.

In relation to the obtained values, alcoholic drinks show higher concentrations of all analytes, as expected from the extractive quality of ethanolic solutions, which favors EDs migration [41]. Although soft drinks have lower ED levels than alcoholic beverages, they display higher values than water samples. This could be a consequence of their mildly acidic nature, which may promote the migration process. BPA and DEHP levels were below the regulated limits indicated above and, in some samples, DEP and DBP showed concentrations higher than those allowed.

Figures of merit for the proposed method in real samples are shown in Table 2 [33,36]. LOQs are appropriate to measure the analyte concentrations, and RMSEPs suggest a good precision. It is worth noting that such limits were estimated using the highest preconcentration factor employed (e.g. 40), and that sensitivity could be improved if a larger volume of sample was processed.

## 4. Conclusions

The developed methodology made it possible the determination of six endocrine disruptors at part per trillion levels and with minimal sample treatment in a wide variety of beverages. The use of LC with dual detection coupled to chemometric analysis allowed a significant reduction of solvent consumption and run time. Applying MCR-ALS was essential to achieve the required selectivity, resolving the high degree of temporal overlapping between the analytes, and rendering excellent results even in the presence of partially overlapped chromatographic and spectral bands among these compounds and non-trivial amounts of interferents.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2016.06.049.

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